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(C12N 5/10
C12R 1:91)
(C12P 21/02
C12R 1:19)

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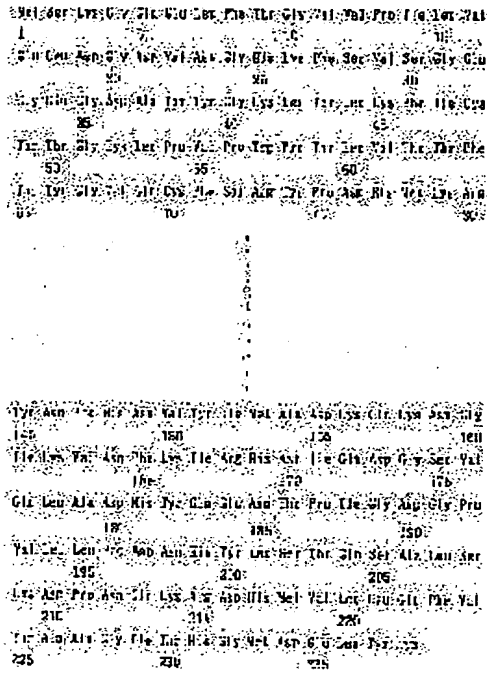
TAKEDA KATSUO
HASEGAWA MAMORU

(54) FLUORESCENT PROTEIN

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a fluorescent protein capable of being expressed even by the culture of a host cell at a high temperature (37°C), emitting stronger fluorescent light than those of conventional fluorescent proteins (GFP), and useful as a labeling agent for the analyses of protein localization in live cells, a reporter for the analyses of promoters, etc., by introducing two mutation amino acids into a wild type GFP.



SOLUTION: This fluorescent protein is obtained by mutating the No. 147 serine and the No. 65 serine of the cDNA of a wild type GFP with proline and threonine, respectively, by a site-specific mutation method, etc., transforming Escherichia coil with a plasmid containing the obtained GFPcDNA and subsequently expressing the mutated GFP containing an amino acid sequence of the formula in the Escherichia coil at a high temperature (37° C). The fluorescent protein emits about three-fold fluorescent light that of S65T mutant,

is contained in a higher concentration than that of the S65T mutant, when expressed in the cell, and emits the fluorescent light under a high temperature (37°C).

LEGAL STATUS

[Date of request for examination]

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[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

* NOTICES *

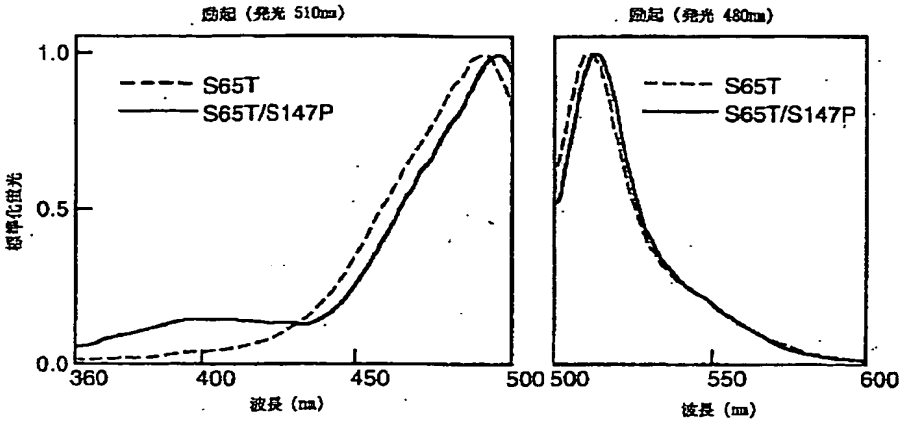
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] Fluorescence protein which includes an amino acid sequence of a publication in an array number 2.
[Claim 2] Fluorescence protein with which 1 or some amino acid include deletion and an amino acid sequence (however, the 65th place is a threonine and the 147th place is a proline) replaced or added in an array number 2 in an amino acid sequence of a publication.
[Claim 3] DNA which carries out the code of the fluorescence protein according to claim 1 or 2.
[Claim 4] A vector containing DNA according to claim 3.
[Claim 5] A vector according to claim 4 characterized by having arranged DNA according to claim 3 on a **-ed promotor's lower stream of a river.
[Claim 6] A host cell holding a vector according to claim 4.
[Claim 7] A manufacture method of fluorescence protein including a process which cultivates a host cell according to claim 6, and collects produced protein according to claim 1 or 2.
[Claim 8] A measuring method of the activity of a **-ed promotor who introduces a vector according to claim 5 into a host cell, and includes a process in which fluorescence emitted from this cell is detected.
[Claim 9] Fluorescence protein according to claim 1 or 2 characterized by uniting with a **-ed amino acid sequence.
[Claim 10] How to detect targetting activity in intracellular [of a **-ed amino acid sequence] which introduces fluorescence protein according to claim 9 into a cell, and is characterized by observing distribution in this intracellular one of this fluorescence protein.
[Claim 11] How to detect targetting activity in intracellular [of a **-ed amino acid sequence] which introduces into a host cell a vector in which DNA which carries out the code of the fluorescence protein according to claim 9 was inserted possible [a manifestation], and is characterized by observing distribution in this intracellular one of this fluorescence protein.

[Translation done.]

Drawing selection drawing 1



[Translation done.]

ITで消化した「S65T/S147P変異体」のcDNAを挿入し、マウス由来のL cellにカルシウム沈殿法で一過的トランスフェクトした。その細胞を37度で48時間培養した後に10%ホルマリンで固定し、蛍光顕微鏡によりノマルスキー(Nomarski)像およびFITCフィルターでの蛍光像(GFPの蛍光)を検出した（図２Ａ下段）。なお、対照として「S65T変異体」のcDNAを用いた（図２Ａ上段）。この結果、「S65T変異体」と比較して、「S65T/S147P変異体」を発現する細胞は、より明るい蛍光像を示した（図２Ａ右下）。

【００３７】また、観察した細胞の内、蛍光を発する細胞の割合、及び細胞の蛍光の強さを測定した（図２Ｂ）。図の横軸は、最も蛍光の強かった細胞の蛍光強度を１とした場合における「S65T/S147P」は「S65T/S147P」は蛍光強度を示し、図の縦軸は、蛍光細胞の細胞数を示す。

【００３８】この結果、「S65T/S147P変異体」のcDNAを挿入された細胞では、対照と比較して、より高い割合で細胞が蛍光を発していた。また、蛍光強度も対照と比較して顕著に高かった。

【００３９】

【発明の効果】本発明により野生型GFPの65番目と147番目のアミノ酸がそれぞれトレオニン、プロリンに置換されたタンパク質が提供された。本発明のタンパク質は、37℃の温度条件下においても蛍光型となり、また従来広く用いられてきた改良型ＧＦＰの約3倍の強い蛍光を発すると共に可溶性タンパク質としての発現量も２倍程度増加しているため、従来のタイプに比べ結果として37℃で約5倍程度明るい蛍光を発することが明らかとなった。この改良型GFPは従来のものに比べ37℃での変が顕著であること、微生物のみならず動物細胞でも適用可能であることから、特に動物細胞や幅広い温度で生育可能な酵母などに有効と考えられる。本発明のGFPは、タンパク質の標識として用い、生細胞における分子の局在を観察する目的に適しているだけでなく、プロモーター解析におけるレポータータンパク質として、またタンパク質の高次構造変化のマーカーとしても有効と考えられ、今後広く細胞生物学、遺伝子工学分野においての利用が期待される。

【００４０】

【配列表】

配列番号： 1
配列の長さ： 717
配列の型 ：核酸
鎖の数 ：二本鎖
トポロジー：直鎖状
配列の種類： cDNA to mRNA
配列の特徴
特徴を表す記号： CDS
存在位置： 1..714
特徴を決定した方法： E

配 列

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT      48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1               5                  10                  15
GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG      96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30
GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC     144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45
ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC     192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60
TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG     240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80
CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA     288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95
ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC     336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT     384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
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11 12

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      115                 120                 125
GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC     432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
  130                 135                 140
TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA     480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160
ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT     528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175
CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT     576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190
GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG     624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205
AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA     672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220
ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA             714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
TAA                                                                 717
```

配列番号： 2　　＊トポロジー：直鎖状

配列の長さ： 238　　配列の種類： タンパク質

配列の型：アミノ酸　　＊

配 列

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1               5                  10                  15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140
Tyr Asn Pro His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
```

13 14

180 185 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

配列番号： 3
配列の長さ： 717
配列の型：核酸
鎖の数：二本鎖
トポロジー：直鎖状

＊配列の種類： cDNA to mRNA
配列の特徴
特徴を表す記号： CDS
存在位置： 1..714
＊ 特徴を決定した方法： E

配 列

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT      48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1               5                  10                  15
GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG      96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30
GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC     144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45
ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC     192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60
ACT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG     240
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80
CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA     288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95
ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC     336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT     384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125
GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC     432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140
TAT AAC CCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA     480
Tyr Asn Pro His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160
ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT     528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175
CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT     576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190
GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG     624
```

15 16

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA 672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA 714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

TAA 717

配列番号： 4 10＊鎖の数：一本鎖
配列の長さ： 36 トポロジー：直鎖状
配列の型：核酸 ＊ 配列の種類：他の核酸 合成DNA

配 列

GGGCCCGGAT CCATGAGTAA AGGAGAAGAA CTTTTC 36

配列番号： 5 ※鎖の数：一本鎖
配列の長さ： 39 トポロジー：直鎖状
配列の型：核酸 ※ 配列の種類：他の核酸 合成DNA

配 列

GGGCAGGGTA CCTTATTTGT ATAGTTCATC CATGCCATG 39

配列番号： 6 20★鎖の数：一本鎖
配列の長さ： 31 トポロジー：直鎖状
配列の型：核酸 ★ 配列の種類：他の核酸 合成DNA

配 列

TTCACCCGGG ATGAGTAAAG GAGAAGAACT T 31

配列番号： 7 ☆鎖の数：一本鎖
配列の長さ： 33 トポロジー：直鎖状
配列の型：核酸 ☆ 配列の種類：他の核酸 合成DNA

配 列

GCACGAATTC TATTTGTATA GTTCATCCAT GCC 33

【図面の簡単な説明】

【図１】「S65T/S147P変異体」及び「S65T変異体」の励起・蛍光スペクトルの測定結果を示す図である。

【図２】図２Ａは、「S65T/S147P変異体」及び「S65T変異体」のcDNAが導入された細胞を蛍光顕微鏡により検出◆し、そのノマルスキー像及び蛍光像を示す顕微鏡写真である。図２Ｂは、被検細胞の中で蛍光を発する細胞の割合及びその細胞の蛍光の強さの測定結果を示す図である。

【図１】